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(54) Preparation of aerosol compositions

(57) The invention relates to the preparation of aerosol compositions.

The invention povides a method for the preparation of liposomes in which at least two separate components are brought together under pressure, a first component comprising water and a second component comprising a lipid material. The components are then passed as a mixture under pressure through a nozzle or other arrangement to produce an aerosol spray containing liposomes. In addition, at least one of the first or second components preferably includes a separate active material.

The invention also provides a pack for use in preparing a liposomal aerosol comprising at least a first and a second chamber, one chamber containing a first component comprising water and the other chamber containing a second component comprising a lipid material, and one or both of the chambers and/or a third chamber including a propellant material. The pack also includes an arrangement for dispensing as a spray a mixture of the first and second components fed from their respective chambers under pressure developed by the propellant material or materials.

GB 2 145 107 A

SPECIFICATION

Preparation of aerosol compositions

The present invention relates to the preparation of aerosol compositions, and in particular to the *in situ* preparation of liposomes using a pressurised aerosol system.

Certain therapeutically-active compounds are administered to patients by inhalation therapy, that is they are administered to the respiratory tract through the nose or mouth as a vapour or gas, or as a mist together with a carrier material in the form of a vapour or gas. Similarly, in certain treatments of the skin, for example, for the treatment of open wounds or sprains, for the treatment of dermatological conditions, or for cosmetic pruposes, it may be desirable to administer an active compound by spraying as a gas or vapour or mist. In either event, it is desirable that as high a proportion as possible of the intact active compound should reach the intended site of action, and sometimes then that active compound should for a time remain intact at the intended site of action. That is to say that the active compound should be administered without breaking down either in transit or immediately on contact with the patient.

Thus, for example, in the administration of the anti-asthmatic bronchodilator compound known as bitolterol (disclosed in British Specification No. 1,298,771) it is desirable to administer the compound in such a manner as to obtain maximum penetration of the bronchi, thereaby to obtain the most effective levels of active compound at the necessary sites of action.

In the past, it has been proposed to produce liposome compositions containing biologically-active compounds and such compositions are disclosed, for example, in British Specifications Nos. 1,575,343, 1,575,344 and 2,013,609 A. However, in such known compositions the liposomal material is one which is pre-prepared and dried, typically by lyophilisation (freeze-drying). The liposomal material is then reconstituted by mixing with water.

Also, aerosol compositions containing a variety of cosmetic and therapeutic materials are known – see, for example, British Specifications Nos. 780,885, 993,702, 1,302,671, 1,381,184, 1,516,195 and 2,001,334 A. However, while such known aerosol compositions may contain lipid ingredients, those ingredients are invariably present merely as dispersing, suspending, emulsifying or like agents. Furthermore, such compositions are intended to produce either a dry spray, so that no liposomal formation is possible or, as in the case of the foundation cream of Specification No. 780,885, are presented as an emulsified premix of any lipid and water.

In addition, as disclosed in U.S. Specification No. 3,594,476, it has been proposed to prepare lecithin aerosols useful for the treatment of lung disorders, which optionally may contain other therapeutic agents such as antibiotics. In the disclosed method of preparation, there is first prepared a suspension of DL dipalmitoyl-α-lecithin in water (or saline solution), with any medicament being dissolved in the water or saline, that suspension then being nebulized i.e. formed into an aerosol, in an ultrasonic generator supplied with a carrier air stream. Thus, the disclosed method relies on relatively expensive ultrasonic equipment to produce the necessary final mixture of lecithin and water, as well as on the use of an initial mixture comprising lecithin suspended in water. Also, ultrasonic equipment can be noisy and would be unsuitable for use on a general basis.

Furthermore, as disclosed in U.S. Specification No. 4,371,451, it has been proposed to produce lecithin-based cookware surface release compositions comprising water, lecithin and dimethylether as propellant. While in that disclosure the solutions are dispensed from an aerosol container, again, the disclosed method relies on the use of an initial mixture of lecithin dispersed in water. Furthermore, what is sought is a quick-drying and thin coating of lecithin which does not foam, rather than a liposomal material as such.

We have now found surprisingly that by using a pressurised aerosol system, liposomal material can be formed extemporaneously from separate aqueous and lipid components, and that active materials of various kinds may be administered with enhanced effect by dispensing them as a sprayed mixture with a liposomal carrier formed *in situ* as the spray mixture is dispensed. Also, such liposomal material formed *in situ* as a spray mixture is dispensed is useful *per se*, for example, in the treatment of lung disorders as known in the art.

Accordingly, the present invention provides a method for the preparation of liposomes, which method comprises bringing together under pressure at least two separate components, a first component comprising water and a second component comprising a lipid material, and passing the mixture under pressure through a nozzle or other arrangement thereby to produce an aerosol spray containing liposomes.

The method of the invention provides for the *in situ* or extemporaneous preparation of liposomes using a pressurised aerosol system. In the invention, an aqueous first component and a second component comprising dry lipid material are mixed to produce a spray in which the liposomes may be present as such or as a liposomal carrier for one or more other materials. Thus, in one preferred embodiment of the invention there is produced a liposomal aerosol

including liposomal carrier and a separate active material which generally will be one which is biologically-active and/or useful in the treatment or care of the human or animal body. Such an active material also will generally be a non-lipid material, although in some instances a lipid may be used to carry an active moiety, for example, attached thereto.

Alternatively, the invention can be used to prepare and provide a liposomal aerosol as such, that is without a separate active compound or other material. Such aerosols would find application in a number of ways, either to prepare liposomes *per se* for a variety of applications, or in therapy. In particular, they would be useful in parturition where newborn infants with breathing difficulties (respiratory distress syndrome) could be assisted to breathe more normally by administering a lipid material to their bronchial regions to supplement the low levels of naturally present lung surfactant found in these infants.

Preferably, however, a separate active material is provided, which initially may be present in either the first or more preferably the second component. Such an active material may be present as a suspension e.g. of say a dry powder, in the first or second component, but preferably is in solution in one of the components. By using a solution of active material it is possible to avoid any settling out as would be the case with a suspension, and any consequent variation in dosage level due to settling.

In the method of the invention, the action of forming/or spraying the mixture leads to the preparation of a liposomal material. Thus, we believe that as the mixture is formed there is produced an emulsion comprising lipid micelles in suspension in water, which then produces liposomes on spraying. Furthermore, where an active material is present the liposomal material includes active material carried by an aqueous medium either trapped in encapsulated form between the lipid layers or sandwiched between molecules of lipid. By varying the conditions under which the two components are mixed and/or sprayed, the size of the liposomes and the relative proportions of free and incorporated active material can be varied within wide limits, the latter up to about 100% incorporation. Thus, for example, by forming and/or spraying the aerosol mixture under varying degrees of mixing activity, for example, as provided by turbulent conditions created say in a mixing chamber and/or feed means associated therewith and/or in a spray nozzle, a spray can be formed either as one having a high proportion of free active material–giving fast action with a reserve of slow-release active material – or as one having a lower proportion of free active material – giving a slower action with a more prolonged and controlled release of active material. Thus, in the latter case, there may be produced a composition comprising active material in which a high proportion of the material is enabled to be carried in a "protected" form to the intended site of action, and/or otherwise to act in a sustained-release manner.

The method of the invention is applicable to the preparation, in a form suitable for immediate administration, of compositions comprising a wide variety of biologically-active meterials and/or other materials useful in the treatment or care of the human or animal body. Typically, the method may be employed to prepare compositions comprising therapeutically-active compounds, including those used in veterinary treatments, as well as biologically-active reagents, cosmetically-active materials, compounds having nutritional value, and other materials used to treat or care for the human or animal body.

Suitable cosmetically-active materials include products intended for skin care and hair care, for example, humectants, artificial tanning agents (optionally in association with colourants), water-soluble anti-sunburn agents, antiperspirants, deodorants, astringents and freshening, toning, cicatrisant, keratolytic and depilatory products, perfumed water, extracts of animal or plant tissues, water-soluble hair dyes, anti-dandruff agents, anti-seborrhoea agents, oxidising agents e.g. bleaching agents, and reducing agents.

Therapeutically-active compounds which may be mentioned include vitamins, steroids, hormones, active peptides, enzymes, vaccines, anti-inflammatory agents, antibiotics and bactericides. However, the method of the invention is particularly applicable to the administration of therapeutically-active compounds used in inhalation therapy such as bronchodilators and anti-asthmatic compounds, as well as anti-tumour agents. Such compounds included in or with the liposomal carrier are able to penetrate the bronchial tree, thus enabling the effectiveness of the therapy to be advantageously modified. Similarly, in the treatment of skin wounds or other dermatological treatments, or in cosmetic treatment, the liposomal carrier can be employed to produce (to advantage) a modified effect, for example, a sustained-release effect, thus enabling a particular level of treatment to be maintained over a longer period of time.

In accordance with the above, and by way of example only, the following specific compounds and routes of administration may be mentioned:

Active Compound	Class of Compound	Route of Administration
Stanozolol	Anabolic Steroid	Topical
Hydrocortisone (and its esters)	Anti-inflammatory steroid	Topical
Betamethasone (and its esters e.g. its valerate)	Anti-inflammatory steroid	Topical
Bitolterol (and its esters e.g. its mesylate)	Catecholamine bronchodilator	Inhalation
Salbutamol	Catecholamine bronchodilator	Inhalation
Theophylline	Xanthene bronchodilator	Inhalation
Sodium chromoglycate	Non-steroidal anti-asthmatic	Inhalation
N-Acetylmuramyl-L-alanyl-D-isoglutamine (adjuvant dipeptide)	Macrophage activating agent (Anti-tumour dipeptide)	Inhalation/ intranasal
Propranolol	B-blocking agent and contraceptive	Intravaginal

Of course, it will be understood by those skilled in the art that a wide variety of other active materials may be employed, as well as other routes of administration. For example, compositions produced in the method of the invention containing appropriate active materials could be administered direct say to the eye or even intravenously.

In addition, there may be mentioned biologically-active materials which may also be therapeutically active such as vaccines, or other biologically-active materials useful as reagents such as antigens and/or antibodies e.g. anti-rabbit IgG. Reagents of this kind may be useful as markers in say testing blood samples, typically for the presence or absence of disease.

The liposomal material produced in the invention may be one which is positively or negatively charged or one which is neutral. The material comprises particulate lipid material existing in aqueous dispersion or suspension, and may include one or more other active materials. Furthermore, the lipid material may be in the form of unilamellar and/or multilamellar lipid bilayers which may carry associated with them any said other active material.

The liposomal material typically comprises a phospholipid, and is preferably a phospholipid which comprises a phosphatidyl choline, e.g. dipalmitoyl phosphatidyl choline as such or in the form of lecithin; a phosphatidyl serine. The liposomal material may be formed from a phospholipid alone or together with cholesterol and/or at least one compound which can confer charge on the liposomal material. Thus, for example, charge may be conferred on the liposomal material by employing dicetyl phosphate or phosphatidic acid, which can give a negatively charged lipid material, or stearylamine, which can give a positively charged lipid material.

The use of the above materials and their proportions, as well as the wide choice of materials and the variations in proportions which may be employed, is well known to the man skilled in the art. However, by way of example only, in one preferred embodiment in accordance with the invention, the second component may comprise a phospholipid consisting of a phosphatidyl choline and cholesterol, typically in relative molar proportions of about 8 : from about 1 to about 2, e.g. about 8.5 : 1.

In another preferred embodiment in accordance with the invention, the second component may comprise a phospholipid consisting of a phosphatidyl choline, cholesterol and dicetyl

phosphate, typically in relative molar proportions of about 8 : from about 1 to about 2 : from about 1 to about 0.05, e.g. about 8.5 : 1 : from about 0.7 to about 0.07.

In a further preferred embodiment in accordance with the invention, the second component may comprise a phospholipid consisting of a phosphatidyl choline, cholesterol and phosphatidic acid, typically in relative molar proportions of about 8 : from about 1 to about 2 : from about 1 to about 0.1, e.g. about 8.5 : 1 : about 0.1.

In yet another preferred embodiment in accordance with the invention, the second component may comprise a phospholipid consisting of a phosphatidyl choline, cholesterol and stearylamine, typically in relative molar proportions of about 8 : from about 1 to about 2 : from about 1 to about 0.05, e.g. about 8.5 : 1 : about 0.06.

Alternatively, the second component may comprise, for example:

a mixture of a phosphatidyl choline e.g. lecithin, and phosphatidic acid e.g. in a ratio of about 60 : about 1; or

a mixture of a phosphatidyl choline e.g. lecithin, and dicetyl phosphate e.g. in a ratio of about 15 : about 1.

The aqueous liposomal material produced in the invention should preferably have a pH value within the physiological range, and thus preferably in the range of from about 6.8 to about 7.4, more preferably of from about 7 to about 7.4. To that end it may also contain a buffer or buffering system in an amount appropriate to establish and maintain the desired pH, and very suitable for that purpose is a phosphate buffer, which typically may comprise sodium dihydrogen phosphate and disodium hydrogen phosphate. Furthermore, such a buffer preferably may be included in the aqueous first component. However, in some instances e.g. where the active material comprises a reagent such as a test marker, the pH may be outside the physiological range.

Depending on the ingredients of the second component, it may be necessary or preferred to include a physiologically-acceptable liquid vehicle, preferably one in which both the lipid material and any other active material are soluble. In the respect, preferred liquid vehicles are relatively volatile organic solvents, for example, ethanol, chloroform (subject to local regulations), ether and isopropyl alcohol.

Furthermore, the first component also may include a physiologically-acceptable liquid vehicle in addition to water. Such a vehicle may be a volatile organic solvent, preferably a water-miscible organic solvent, of which ethanol is preferred.

In a preferred embodiment of the invention, the first and second components are mixed under pressure by the use of one or more aerosol propellants, which may be an ingredient of one or both components and/or supplied separately. Preferably, however, a propellant is an ingredient of the second component, and pressure is applied to the first component via a propellant held separate therefrom.

Typically, one or each propellant may comprise a hydrocarbon or halogenated hydrocarbon or mixture of such hydrocarbons. Examples of such propellants are propane, butane, dimethylether, and propellant 11 (trichlorofluoromethane), although dichlorodifluoromethane or dichlorotetrafluoroethane or mixtures thereof are preferred. The propellant may be one formulated as a low pressure propellant developing pressures up to say from about 25 to about 30 psi such as a 20:80 w/w mixture of dichlorodifluoromethane and dichlorotetrafluoroethane, or the propellant may be a relatively high pressure propellant developing pressures up to say about 70 psi such as an 80:20 w/w mixture of dichlorodifluoromethane and dichlorotetrafluoroethane. It will be appreciated, of course, that relative dose rates as between components can be controlled and varied *inter alia* depending on the propellant pressure employed for each component.

In the method of the invention it is preferred that the first and second components should each be provided isolated from each other, that each of the components should be supplied in controlled discrete doses to a mixing chamber under their own propellant pressure, and that the thus-formed mixture should be discharged from the mixing chamber through an orifice under pressure from at least one of said propellants. Preferably also, the first and second components are mixed in a manner which provides a metred dosage of any said active material.

More preferably, the second component is supplied as a metered dose to a metered dose of the first component, and is preferably brought into contact with at lest the bulk of the first component at a plurality of sites within said metered dose. Thus, in particular, the method of the invention more preferably comprises in sequence the following steps, namely:

the first component is supplied to a mixing chamber in a metered amount;

a metered amount of the second component is supplied to the mixing chamber in a manner to produce turbulent mixing of the two components, preferably by bringing the second component into the mixing chamber at a plurality of sites; and

the mixture is discharged from the mixing chamber.

Conveniently, the metered amount of first component is controlled by supplying the first component to the mixing chamber in an amount to fill the chamber, and by choosing the chamber volume so as to give the desired metered amount of first component.

The invention also includes a pack for use in preparing a liposomal aerosol, which pack comprises at least a first and a second chamber, one chamber containing a first component comprising water, which may be a buffered aqueous material, and the other chamber containing a second component comprising a lipid material. one of the components optionally including, preferably in suspension or solution therein, a said active material e.g. for use in the treatment or care of the human or animal body, and one or both of the chambers and/or a third chamber including a propellant material, the pack including an arrangement for dispensing as a spray a mixture of the first and second components fed from their respective chambers under pressure developed by the propellant material or materials.

Preferably, the dispensing arrangement is such that the mixing ratio of the components is controlled (say to a metered level or to within a narrow metered range of ratios), whereby the desired metered dosage of any said active material can be dispensed.

Once again, it is preferred that the active material should be in suspension or solution in the second component comprising a lipid material, and more preferably the active compound should be in solution, in order to avoid settling. Generally, the active material, the first and second components, and the propellant are as described above for the method of the invention.

Preferably, the pack of the invention includes a discharge orifice, and a mixing chamber and valve means to permit said first and second components to be supplied from their respective chambers under pressure developed by one or more propellants into the mixing chamber and to control the ratio of components (say to a metered level or within a narrow metered range of ratios), whereby the desired metered dose of mixture i.e. active material plus liposome carrier or liposome *per se*, can be dispensed through the discharge orifice.

Preferably, said valve means comprises an arrangement (say of two valves) to permit in sequence the first component to be supplied to the mixing chamber to fill the chamber, a metered dose of the second component to be supplied to the mixing chamber in a manner to produce turbulent mixing of the two components, and discharge of the mixture through said discharge orifice. Preferably also, the valve means includes an arrangement to permit the second component to be supplied to the mixing chamber at a plurality of sites within the chamber.

If desired, there may also be provided additional mixing means either upstream or downstream of the mixing chamber to create additional intimate and/or turbulent mixing conditions to promote emulsion formation prior to discharge through the discharge orifice. Thus, for example, the discharge orifice arrangement may itself include a subsidiary mixing chamber into which the mixture is fed in such a manner as to create additional turbulent conditions e.g. by feeding tangentially into the chanmber via one or a plurality of inlets, before discharge.

In the pack of the invention, the first chamber may be a first aerosol can or bag, the second chamber may be a second aerosol can or bag, and the cans or other first and second closed chambers may be arranged:

End-to-end with the respective valve means and mixing chamber disposed between or above the cans or other chambers;

Side-by-side with the respective valve means and mixing chamber disposed between or above the cans or other chambers; or

The first within the second (or vice versa) with the valve means and mixing chamber disposed within or above the outer can or other chamber. Aerosol containers using this kind of arrnagement are disclosed, for example, in U.S. Specifications Nos. 3,325,056 and 3,326,416.

In the pack of the invention there may be provided an outer housing e.g. cannister, within which the closed chambers may be disposed and through which the valve means may be operated. In such a pack, it is preferred that the second components includes a propellant and is disposed in an inner rigid-walled closed chamber, the first component is disposed in an outer flexible-walled closed chamber, preferably surrounding the inner chamber, the housing is a rigid walled housing and the space between the outer closed chamber and the surrounding housing includes a propellant. An especially preferred arrangement of two-component pack suitable for providing those features is that disclosed in European Patent Application No. 84-10-4589.1, and British Patent Application No. , both based on Swiss Patent Application No. 4450/83. However, if desired the inner chamber arrangement may be the same as the outer chamber arrangement, that is with the inner chamber being formed as a flexible-walled chamber surrounded by a rigid-walled chamber containing propellant.

As will be understood from the foregoing description, in putting the present invention into effect, the dose level of active material dispensed by each operation of the above-described pack may be varied widely depending on a variety of factors, in particular:

1. The concentration of active material in the first and/or second component; and
2. The mixing ratio of the first and the second components.

In the case of the mixing ratio of the components, that will depend on the size of the metered amounts of the first and the second components supplied for mixing, which in turn will depend on the particular mixing and/or valve arrangements employed. In the particular case of the

especially preferred arrangement mentioned above, the mixing chamber preferably has a volume of from about 50 to about 100 e.g. 75 to 100, microlitres, and the valve controlling the metering of the second component preferably dispenses to the mixing chamber from about 50 to about 100 microlitres per activation.

In the case of liposome size, we believe that will be controlled in the main by the pressure generated by the propellant or propellants and/or by the viscosity of the components. Generally speaking, the more vigorously the compounds are mixed the smaller will be the size of liposomes. However, the size of the discharge or dispensing orifice of the valve used to dispense the mixed components may also effect the size of liposomes produced, and preferably such a discharge orifice has a size of from about 350 to about 450 microns.

On the other hand, the above-mentioned parameters once having been fixed, the resultant dose level can readily be ascertained, as can also the degree of incorporation of any active material, and the size of liposomes produced.

As will be appreciated by those familiar with formulating active materials with carrier materials, in the present invention the level of incorporation of active material within the liposomal carrier may have a pronounced effect on the datum dose level chosen for the overall formulation. Accordingly, such a datum dose level will be a matter of formulating choice depending on the envisaged criteria to be met by any one particular formulation.

The invention further includes a liposomal material or a liposomal composition when prepared as a spray by a method as described herein or by the use of a pack as described herein.

The following Examples illustrate the method and aerosol pack of the invention.

In the specific Examples given, a majority of the formulations include ammonium molybdate as an electron dense material, which was incorporated merely as a marker to facilitate detection of the liposomes, and measurement of their diameter using an electron microscope. It will, of course, be understood by those familiar with pharmaceutical formulations that ammonium molybdate, which is moderately toxic, should not be part of an actual working formulation for administration to a patient. As can be seen from a comparison say of Example 19 and Example 20 below, the omission of ammonium molybdate has no detrimental effect on the level of incorporation of active ingredient and, in fact, the level of incorporation is slightly higher in Example 20. Accordingly, in any of the exemplified formulations the molybdate marker can be omitted without detriment, and the given results can be obtained in its absence.

Also, while a variety of levels of active ingredient are exemplified, and while those in turn give a variety of levels of free and incorporated active ingredient per actuation, again it will be understood by the skilled formulator that the dose level and the kind of formulation chosen for any particular working pack will depend on the particular desired dosing regimen to be achieved, as well as any necessary or desired balance between unincorporated and incorporated active material.

For example, assuming an average person on average applies a cream formulation to skin at a level of 2.2 mg/cm^2, and assuming a single aerosol actuation or "shot" covers 25 cm^2 of skin, the following comparison may be made between exemplified and standard compositions:

Formulation	Application level (micrograms/cm^2)
Stanozolol cream (0.1%)	2.3
Stanozolol aerosol of Example 21	4.0
Hydrocortisone cream (1.0%)	23
Hydrocortisone aerosol of Example 25	4.8
Betamethasone cream (0.1%)	2.5
Betamethasone aerosol of Example 27	0.64

Thus, the active material can be dispensed at an overall dose level above or below (or even at) the known standard level, to provide any desired variation in effect, that variety of effect being enhanced by the degree of incorporation chosen.

Similarly, in the case of formulations destined for inhalation, the overall dose level may be varied depending on the required effect. Thus, for example, as illustrated by Example 31, salbutamol may be dispensed per actuation effectively as 125 micrograms of free material, as compared with 100 micrograms per actuation from a standard dispenser. Furthermore, as illustrated by Examples 29 and 30 respectively, active material can be administered at much higher or much lower dose levels per actuation than is standard – that is 933 micrograms vs. 200 micrograms (standard) in the case of bitolterol in Example 29, and 200 micrograms vs.

2mg (standard) in the case of sodium cromoglycate in Example 30 – although in both instances the dose level may be adjusted to the standard level by altering the overall amount of active material carried by the second or first component respectively.

EXAMPLE 1

240 mg of egg lecithin and 28 mg of cholesterol were dissolved in 4 ml of ethanol and the solution added to 25 g of a 20:80 w/w mixture of dichlorodifluoromethane and dichlorotetrafluoroethane propellant. The mixture was then sealed in a first container fitted with a valve. 25 ml of a 3.3 mM aqueous sodium phosphate solution, pH 7.2, were introduced into an aluminium bag which was then sealed in a separate container fitted with a valve and containing 25 g of the above propellant. The containers were connected via a mixing chamber and each actuation of the valve mechanism released an aliquot of the lipid solution and an aliquot of the phosphate solution into the mixing chamber, and thence to the outside atmosphere via a narrow orifice. The resulting aerosol contained multilamellar and unilamellar liposomes with a mean diameter of from 40 nm to 1000 nm.

EXAMPLE 2

The procedure of Example 1 was repeated except that the first container fitted with a valve contained a mixture of 240 mg of egg lecithin, 28 mg of cholesterol and 4 mg of phosphatidic acid dissolved in 4 ml of ethanol, together with 25 g of a 40 : 60 w/w mixture of dichlorodifluoromethane and dichlorotetrafluoromethane propellant.

The resulting arrangement could be used to produce an aerosol which contained negatively charged multilamellar and unilamellar liposomes with a mean diameter of from 40 nm to 1000 nm.

EXAMPLE 3

The procedure of Example 1 was repeated except that the first container fitted with a valve contained a mixture o 240 mg of egg lecithin, 28 mg of cholesterol and 1.6 mg of stearylamine dissolved in 4 ml of ethanol, together with 25 g of a 20 : 80 w/w mixture of dichlorodifluoromethane and dichlorotetrafluoroethane propellant.

The resulting arrangement could be used to produce an aerosol which contained positively charged multilamellar and unilamellar liposomes with a mean diameter of from 40 nm to 1000 nm.

EXAMPLE 4

The procedure of Example 1 was repeated except that the first container fitted with a valve contained a mixture of 240 mg of egg lecithin and 4 mg of phosphatidic acid dissolved in 4 ml of ethanol, together with 25 g of a 20 : 80 w/w mixture of dichlorodifluoromethane and dichlorotetrafluoroethane propellant.

The resulting arrangement could be used to produce an aerosol which contained negatively charged multilamellar and unilamellar liposomes with a mean diameter of from 40 nm to 1000 nm.

EXAMPLE 5

The procedure of Example 1 was repeated except that the first container fitted with a valve contained a mixture of 24 mg of dipalmitoyl phosphatidyl choline, 2.8 mg of cholesterol, 2 mg of dicetyl phosphate and 2 mg of bitolterol mesylate dissolved in 4 ml of ethanol, together with 25 g of a 40 : 60 w/w mixture of dichlorodifluoromethane and dichlorotetrafluoroethane propellant.

The resulting arrangement could be used to produce an aerosol which contained negatively charged multilamellar and unilamellar liposomes with a mean diameter of from 40 nm to 1000 nm. In addition, more than 50% of the bitolterol mesylate was incorporated in the liposomes.

EXAMPLE 6

The procedure of Example 1 was repeated except that the first container fitted with a valve contained a mixture of 240 mg of egg lecithin, 28 mg of cholesterol, 2 mg of dicetyl phosphate and 20 mg of bitolterol mesylate dissolved in 4 ml of ethanol, together with 25 g of a 40 : 60 w/w mixture of dichlorodifluoromethane and dichlorotetrafluoroethane propellant.

The resulting arrangement could be used to produce an aerosol which contained multilamellar and unilamellar liposomes with a diameter of from 40 nm to 100 nm. In addition, more than 50% of the bitolterol mesylate was incorporated in the liposomes.

EXAMPLE 7

50 mg of egg lecithin were dissolved in ethyl alcohol (96% BP) and the volume made up to 1.0 ml with ethyl alcohol to provide a lipid component. The resultant solution was transferred to

a glass vessel and 7.0 g of dichlorodifluoromethane were added thereto. The glass vessel was then sealed with a 65 microlitre metering valve associated with a 75 microlitre mixing chamber arranged to receive lipid component discharged through the metering valve and itself forming part of a second valve system.

The thus-formed assembly was placed within a second flexible vessel (a tubular bag) containing 40 ml of ammonium molybdate solution (0.5% w/v in deionised water) to provide an aqueous component, that component being in communication with the mixing chamber with the second valve in its closed position. The second vessel was then crimped into a cannister containing 2.0 g of dichlorodifluoromethane propellant in a manner whereby the second valve system (associated with the bag and mixing chamber) could, when itself activated, also activate the first valve for discharge of lipid component into the mixing chamber, and thereby provide discharge of mixed components from the overall aerosol cannister assembly e.g. as described in the above-mentioned British Patent Application No. , based on Swiss Patent Application No. 4450/83. A standard button actuator with 450 micron orifice was then fitted to the second valve system.

The resulting aerosl cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 40 nm to 400 nm.

EXAMPLE 8

The procedure of Example 7 was repeated except that the glass vessel was fited with a 50 microlitre metering valve associated with a 100 microlitre mixing chamber. The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 40 nm to 1000 nm.

EXAMPLE 9

The procedure of Example 7 was repeated except that the glass vessel was fitted with a 100 microlitre metering valve associated with a 100 microlitre mixing chamber. The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes wth diameters of from 50 nm to 1000 nm.

EXAMPLE 10

60.0 mg of egg lecithin and 7.0 mg of cholesterol were dissolved in 1.0 ml of ethyl alcohol (96% BP). The resultant solution was transferred to a glass vessel and 6.0 g of a 20:80 w/w mixture of dichlorodifluoromethane and dichlorotetrafluoroethane were added thereto. The glass vessel was then sealed with a 50 microlitre metering valve associated with a 100 microlitre mixing chamber, as in Example 7.

The thus-formed assembly was placed within a second, flexible vessel containing 40 ml of 3.3 mM aqueous sodium phosphate solution having a pH of 7.2. Again, as in Example 7, the second vessel was then crimped into an aerosol cannister containing 2.0 g of dichlorodifloromethane propellant in a manner whereby the second valve system could provide discharge from the overall aerosol cannister assembly. A standard button actuator with 450 micron orifice was then fitted to the second valve system.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 40 nm to 1000 nm.

EXAMPLES 11 to 15

The procedure of Example 10 was repeated using in the glass vessel a solution of the following ingredients dissolved in 1.0 ml of ethyl alcohol (96% BP), namely:

Example No.

11. 60.0 mg of egg lecithin, 1.0 mg of phosphatidic acid and 7.0 mg of cholesterol.
12. 60.0 mg of egg lecithin, 0.4 mg of stearylamine and 7.0 mg of cholesterol.
13. 60.0 mg of egg lecithin and 1.0 mg of phosphatidic acid.
14. 0.5 mg of bitolterol mesylate, 6.0 mg of dipalmitoylphosphatidyl choline, 0.5 mg of dicetyl phosphate and 0.7 mg of cholesterol.
15. 5.0 mg of bitolterol mesylate. 60.0 mg of egg lecithin. 0.5 mg of dicetyl phosphate and 7.0 mg of cholesterol.

The resulting aerosol cannister assembly for each of Examples 11 to 15 could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 40 nm to 1000 nm.

EXAMPLE 16

100 mg of egg lecithin and 6.0 mg of stanozolol were dissolved in ethyl alcohol (96% BP) and the volume made up to 2.0 ml with ethyl alcohol. The resultant solution was transferred to a glass vessel and 9.0 g of dichlorodifluoromethane were added thereto. The glass vessel was

then sealed with a 50 microlitre metering valve associated with a 100 microlitre mixing chamber, as in Example 7.

The thus-formed assembly was placed within a second, flexible vessel containing 40 ml of ammonium molybdate solution (0.5% w/w in deionised water). Again, as in Example 7, the second vessel was then crimped into an aerosol cannister containing 2.0 g of dichlorodifluoromethane propellant in a manner whereby the second valve system could provide discharge from the overall aerosol cannister assembly. A standard, button actuator with 450 micron orifice was then fitted to the second valve system.

The resulting aerosl canister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 40 nm to 110 nm. Of the 50.0 micrograms of stanozolol released by each actuation of the second valve system, an amount of 21.6 micrograms was shown to be associated with the liposomes.

EXAMPLE 17

The procedure of Example 16 was repeated except that the solution filled into the glass vessel comprised 450 mg of egg lecithin and 60 mg of stanozolol dissolved in ethyl alcohol (96% BP), with the volume made up to 3.0 ml with ethyl alcohol, 4.5 g of dichlorodifluoromethane were added to the vessel, and the button actuator had a 360 micron orifice.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 44 nm to 440 nm. Of the 500.0 micrograms of stanozolol released by each actuation of the second valve system, an amount of 35.4 micrograms was shown to be associated with the liposomes.

EXAMPLE 18

The procedure of Example 17 was repeated except that the metering valve was a 100 microlitre metering valve, and the button actuator had a 450 micron orifice.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 25 nm to 190 nm. Of the 1000.0 micrograms of stanozolol released by each actuation of the second valve system, an amount of 170.5 micrograms was shown to be asociated with the liposomes.

EXAMPLE 19

The procedure of Example 16 was repeated except that the solution filled into the glass vessel comprised 900 mg of egg lecithin and 60 mg of stanozolol dissolved in ethyl alcohol (96% BP), with the volume made up to 3.0 ml with ethyl alcohol, and 4.5 g of dichlorodifluoromethane were added to the vessel.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 40 nm to 350 nm. Of the 500.0 micrograms of stanozolol released by each actuation of the second valve system, an amount of 81.3 micrograms ws shown to be associated with the liposomes.

EXAMPLE 20

The procedure of Example 19 was repeated except that the second vessel contained 40 ml of deionised water only.

Of the 500 micrograms of stanozolol released by each actuation of the second valve system, an amount of 85.0 micrograms was shown to be associated with the liposomes.

EXAMPLE 21

The procedure of Example 20 was repeated except that the solution filled into the glass vessel contained 12 mg of stanozolol.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 40 nm to 350 nm. Of the 100 micrograms of stanozolol released by each actuation of the second valve system, an amount of 76.8 micrograms was shown to be associated with the liposomes.

EXAMPLE 22

The procedure of Example 21 was repeated except that the second vessel contained 40 ml of a solution of ethyl alcohol (1% v/v) in deionised water.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 30 nm to 500 nm. Of the 100 micrograms of stanozolol released by each actuation of the second valve system, an amount of 77.3 micrograms was shown to be associated with the liposomes.

EXAMPLE 23

The procedure of Example 21 was repeated except that the second vessel contained 40 ml of

a solution of ethyl alcohol (5% v/v) in deionised water.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 30 nm to 500 nm. Of the 100 micrograms of stanozolol released by each actuation of the second valve system, an amount of 76.3 micrograms was shown to be associated with the liposomes.

EXAMPLE 24

The procedure of Example 21 was repeated except that the second vessel contained 40 ml of a solution of ethyl alcohol (10% v/v) in deionised water.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 30 nm to 500 nm. Of the 100 micrograms of stanozolol released by each actuation of the system, an amount of 66.6 micrograms was shown to be asociated with the liposomes.

EXAMPLE 25

The procedure of Example 17 was repeated except that the solution filled into the glass vessel comprised 900 mg of egg lecithin and 14.4 mg of hydrocortisone BP dissolved in ethyl alcohol (96% BP), with the volume made up to 3.0 ml with ethyl alcohol.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 44 nm to 220 nm. Of the 120 micrograms of hydrocortisone BP released by each actuation of the second valve system, an amount of 54.0 micrograms was shown to be associated with the liposomes.

EXAMPLE 26

The procedure of Example 16 was repeated except that the solution filled into the glass vessel comprised 841 mg of egg lecithin, 54.8 mg of dicetyl phosphate and 14.4 mg of hydrocortisone BP dissolved in ethyl alcohol (96% BP), with the volume made up to 3.0 ml with ethyl alcohol, and 4.5 g of dichlorodifluoromethane were added to the vessel.

Of the 120 micrograms of hydrocortisone BP released by each actuation of the second valve system, an amount of 45.6 micrograms was shown to be associated with the liposomes.

EXAMPLE 27

The procedure of Example 26 was repeated except that the solution filled into the glass vessel comprised 841 mg of egg lecithin and 4.8 mg of betamethasone valerate BP/USP/NF dissolved in ethyl alcohol (96% BP), with the volume made up to 3.0 ml with ethyl alcohol.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 20 nm to 300 nm. In addition, 16 micrograms of betamethasone valerate were released by each actuation of the second valve system.

EXAMPLE 28

The procedure of Example 27 was repeated except that the betamethasone was omitted from the solution filled into the glass vessel.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 30 nm to 1000 nm.

EXAMPLE 29

The procedure of Example 26 was repeated except that the solution filled into the glass vessel comprised 841 mg of egg lecithin and 112 mg of bitolterol mesylate dissolved in ethyl alcohol (96% BP), with the volume made up to 3.0 ml with ethyl alcohol.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 25 nm to 250 nm. Of the 933 micrograms of bitolterol mesylate released by each actuation of the second valve system, an amount of 545 micrograms was shown to be associated with the liposomes.

EXAMPLE 30

The procedure of Example 28 was repeated except that the second vessel contained 40 ml of a solution of sodium chromoglycate USP XX, BP '80 (2mg/ml) and ammonium molybdate (5mg/ml) in 0.1 M sodium phosphate buffer at a pH of 7.4.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 35 nm to 700 nm. Of the 200 micrograms of sodium chromoglycate released by each actuation of the system, an amount of 22 micrograms was shown to be associated with the liposomes.

EXAMPLE 31

The procedure of Example 18 was repeated except that the 60 mg of stanozolol were replaced by 15 mg of salbutamol base BP.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes with diameters of from 30 nm to 300 nm. Of the 250 micrograms of salbutamol relesed by each actuation of the second valve system, an amount of 125 micrograms was shown to be associated with the liposomes.

EXAMPLE 32

The procedure of Example 28 was repeated except that the second vessel contained 10 ml of a solution of N-acetylmuramyl-L-alanyl-D-isoglutamine [NALAG] (100 micrograms/ml) and ammonium molybdate (5mg/ml) in deionised water.

The resulting aerosol cannister assembly could be used to produce an aerosol spray which contained unilamellar and multilamellar liposomes. In addition, 10 micrograms of NALAG were released by each actuation of the second valve system.

EXAMPLE 33

841 mg of egg lecithin were dissolved in ethyl alcohol (96% BP) and the volume made to 3.0 ml with ethyl alcohol. The resultant solution was transferred to a glass vessel and 4.5 g of dichlorodifluoromethane were added thereto. The glass vessel was then sealed with a 50 microlitre metering valve associated with a 100 microlitre mixing chamber, as in Example 7.

The thus-formed assembly was placed within a second, flexible vessel containing 35 ml of a dilution (50 microlitres/ml) of anti-rabbit IgG (1/64 Titre versus 1/640 normal rabbit serum as determined by agar block precipitin titration) and ammonium molydbate (5 mg/ml) in deionised water. Again, as in Example 7, the second vessel was then crimped into a cannister containing 2.0 g of dichlorodifluoromethane propellant in a manner whereby a second valve system could provide discharge from the overall aerosol cannister assembly. A standard, button actuator with 450 micron orifice was then fitted to the second valve system.

The resulting aerosol cannister assembly could be used to provide an aerosol spray which contained unilamellar and multilamellar liposomes. 5 microlitres of anti-rabbit IgG (1/64 Titre) were released by each actuation of the second valve system.

It is to be understood that the invention is not limited to the details of the above specific Examples. Thus, for example, a variety of other lipid materials may be employed to form the desired liposomal material and a wide range of active materials may be carried therewith. Also, a number of other aerosol arrangements may be employed, particularly one of those other specific arrangements mentioned above. Furthermore, the necessary pressure can be generated by means other than the use of one or more aerosol propellants.

Generally, as will be appreciated from the above description, the invention overall embraces any method for the *in situ* preparation of liposomes using a pressurised aerosol system, whether or not the liposomes include or are with a separate active component, and a pack for use in such a preparation.

CLAIMS

1. A method for the preparation of liposomes, which method comprises bringing together under pressure at least two separate components, a first component comprising water and a second component comprising a lipid material, and passing the mixture under pressure through a nozzle or other arrangement thereby to produce an aerosol spray containing liposomes.

2. A method according to claim 1, wherein at least one of said first or second components includes an active material which is biologically-active and/or useful in the treatment of care of the human or animal body.

3. A method according to claim 2, wherein the active material is in solution in the first or second component.

4. A method according to claim 2 or claim 3, wherein the active material is therapeutically- or cosmetically-active.

5. A method according to claim 4, wherein the active material is a bronchodilator, an anti-asthmatic compound, an anti-tumour agent, an anti-inflammatory, a contraceptive or an anabolic steroid.

6. A method according to claim 4, wherein the active material is a compound selected from bitolterol and its esters, stanozolol, hydrocortisone and its esters, betamethasone and its esters, salbutamol, theophylline, sodium chromoglycate, N-acetylmuramyl-L-alanyl-D-isoglutamine, or propranolol.

7. A method according to claim 2 or claim 3, wherein the active material is a biologically-active reagent.

8. A method according to any one of claims 2, 3 or 7, wherein the active material is an antigen or antibody.

9. A method according to claim 2 or claim 3, wherein the active material is one or more

compounds having nutritional value.

10. A method according to any one of the preceding claims, wherein the lipid material of the second component is a phospholipid material chosen so as to provide a positively charged, a negatively charged or a neutral liposome.

11. A method according to claim 10, wherein the lipid material of the second component is a phospholipid which comprises either a phosphatidyl choline, a phosphatidyl ethanolamine or a phosphatidyl serine alone, or one or more of those lipid materials together with cholesterol and/or at least one of dicetyl phosphate, phosphatidic acid or stearylamine.

12. A method according to claim 11, wherein the second component is chosen to provide a neutral liposome and comprises a phospholipid consisting of a phosphatidyl choline and cholesterol in relative molar proportions of about 8 : from about 1 to about 2.

13. A method according to claim 11, wherein the second component is chosen to provide a negatively charged liposome and comprises a phospholipid consisting of a phosphatidyl choline, cholesterol and dicetyl phosphate in relative molar proportions of about 8 : from about 1 to about 2 : from about 1 to about 0.05.

14. A method according to claim 11, wherein the second component is chosen to provide a negatively-charged liposome and comprises a phospholipid consisting of a phosphatidyl choline, cholesterol and phosphatidic acid in relative molar proportions of about 8 : from about 1 to about 2 : from about 1 to about 0.1.

15. A method according to claim 11, wherein the second component is chosen to provide a positively charged liposome and comprises a phospholipid consisting of a phosphatidyl choline, cholesterol and stearylamine in relative molar proportions of about 8 : from about 1 to about 2 : from about 1 to about 0.05.

16. A method according to any one of claims 11 to 15, wherein the phosphatidyl choline is dipalmitoyl phosphatidyl choline as such or in the form of lecithin.

17. A method according to any one of the preceding claims, wherein the first component includes a buffer or buffering system in an amount appropriate to establish and maintain a pH within the physiological range.

18. A method according to any one of the preceding claims, wherein the second component includes a physiologically-acceptable liquid vehicle.

19. A method according to claim 18, wherein the vehicle is volatile organic solvent in which both the lipid material and any said active material are soluble.

20. A method according to claim 18, wherein the liquid vehicle is ethanol, chloroform, ether or isopropyl alcohol.

21. A method according to any one of the preceding claims, wherein the first component includes a physiologically-acceptable liquid vehicle in addition to water.

22. A method according to claim 21, wherein the vehicle is a volatile, organic solvent.

23. A method according to claim 22, wherein the solvent is ethanol.

24. A method according to any one of the preceding claims, wherein the first and second components are mixed under pressure produced by at least one aerosol propellant.

25. A method according to claim 24, wherein a propellant is an ingredient of one or both components.

26. A method according to claim 25, wherein a propellant is an ingredient of the second component, and pressure is applied to the first component via a propellant held separate therefrom.

27. A method according to any one of claims 24 to 26, wherein one or each propellant comprises a hydrocarbon, a halogenated hydrocarbon or a mixture of halogenated hydrocarbons.

28. A method according to any one of claims 24 to 27, wherein one or each propellant is dichlorodifluoromethane.

29. A method according to any one of claims 24 to 27, wherein one or each propellant is a low pressure propellant developing pressures up to from about 25 to about 30 psi.

30. A method according to claim 29, wherein one or each propellant is a 20 : 80 w/w mixture of dichlorodifluoromethane and dichlorotetrafluoroethane.

31. A method according to any one of claims 24 to 27, wherein one or each propellant is a high pressure propellant developing pressures up to about 70 psi.

32. A method according to claim 31, wherein one or each propellant is an 80 : 20 w/w mixture of dichlorodifluoromethane and dichlorotetrafluoroethane.

33. A method according to any one of the preceding claims, in which said first and second components are each provided isolated from each other, each of the components are supplied in controlled discrete doses to a mixing chamber under their own propellant pressure, and the thus-formed mixture is discharged from the mixing chamber through an orifice under pressure from at least one of said propellants.

34. A method according to any one of claims 2 to 33, wherein the first and second components are mixed in a manner which provides a metered dosage of the said active material.

35. A method according to any one of the preceding claims, wherein the second component

is supplied as a metered dose to a metered dose of the first component.

36. A method according to any one of claims 33 to 35, wherein in sequence the first component is supplied to the mixing chamber to fill the chamber, a metered dose of the second component is supplied to the mixing chamber in a manner to produce turbulent mixing of the two components and the mixture is discharged from the mixing chamber.

37. A method according to claim 1 and substantially as hereinbefore described with reference to any one of the specific Examples.

38. A pack for use in preparing a liposomal aerosol, which pack comprises at least a first and a second chamber, one chamber containing a first component comprising water and the other chamber containing a second component comprising a lipid material, and one or both of the chambers and/or a third chamber including a propellant material, the pack including an arrangement for dispensing as a spray a mixture of the first and second components fed from their respective chambers under pressure developed by the propellant material or materials.

39. A pack according to claim 38, wherein at least one of said first or second components includes an active material which is biologically-active and/or useful in the treatment or care of the human or animal body.

40. A pack according to claim 39, wherein the dispensing arrangement is such that the mixing ratio of the components is controlled whereby the desired metered dosage of the said active material can be dispensed.

41. A pack according to any one of claims 38 to 40, which also includes a discharge orifice, and a mixing chamber and valve means to permit said first and second components to be supplied from their respective chambers under pressure developed by one or more propellants into the mixing chamber and to control the ratio of components, whereby the desired metered dose of mixture can be dispensed through the discharge orifice.

42. A pack according to claim 41, wherein said valve means comprises an arrangement to permit in sequence the first component to be supplied to the mixing chamber to fill the chamber, a metered dose of the second component to be supplied to the mixing chamber in a manner to produce turbulent mixing of the two components, and discharge of the mixture through said discharge orifice.

43. A pack according to claim 41 or claim 42, wherein the valve means includes an arrangement to permit the second component to be supplied to the mixing chamber at a plurality of sites within the chamber.

44. A pack according to any one of claims 41 to 43, wherein the first and second chambers are closed chambers arranged end-to-end with the respective valve means and mixing chamber disposed between their ends.

45. A pack according to any one of claims 41 to 43, wherein the first and second chambers are closed chambers arranged side-by-side with the respective valve means and mixing chamber disposed between or above the chambers.

46. A pack according to any one of claims 41 to 43, wherein the first and second chambers are closed chambers arranged one within the other, the valve means and mixing chamber being disposed within and/or above the outer chamber.

47. A pack according to any one of claims 41 to 46, which includes an outer housing within which the chambers are disposed and through which the valve means may be operated.

48. A pack according to claims 46 and 47, wherein the second component includes a propellant and is disposed in an inner rigid-walled closed chamber, the first component is disposed in an outer flexible-walled closed chamber, the housing is a rigid-walled housing and the space between the outer closed chamber and the surrounding housing includes a propellant.

49. A pack according to any one of claims 38 to 48, wherein the first and second components, the propellant and any said active material are as defined in any one of claims 3 to 32.

50. A pack according to claim 38, and substantially as hereinbefore described.

51. A pack according to claim 38, and substantially as hereinbefore described with reference to any one of the specific Examples.

52. Liposomes or a liposomal composition when prepared as a spray by a method according to any one of claims 1 to 37 or by the use of a pack according to any one of claims 38 to 51.

53. A method for the *in situ* preparation of liposomes, whether or not the liposomes include or are with a separate active material, using a pressurised aerosol system, and a pack for use in such preparation, substantially as hereinbefore described or defined.

54. Each and every novel method for the preparation of liposomes, dispensing of active material, or pack therefor, as hereinbefore described or defined.

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